A Research Note

Determination of Safrole and Isosafrole in Ham by HPLC with UV Detection

M.P. ZUBILLAGA and G. MAERKER

ABSTRACT

An improved method has been developed for the determination of safrole and isosafrole in meat. To ham were added known amounts (5-100 ppm) of either compound or mixtures of both. The samples were extracted with hexane/ethyl acetate (95/5, v/v) by a dry column procedure, and the eluate was analyzed by HPLC with UV detection. Recoveries ranged from 91–102% for the individual components and from 93–100% for mixtures. The improved method requires less analysis time and instrumentation and is more sensitive than a previous method.

INTRODUCTION

SAFROLE, a major constituent of oil of sassafras (Guenther, 1949) and isosafrole, its positional isomer, are prohibited food additives (Anonymous, 1960). Concern was expressed by the Food Safety and Inspection Service (FSIS), USDA, that the possible use by some meat processors of sassafras wood in a smoking process might result in the presence of safrole or isosafrole in some meat products, particularly smoked ham. We are reporting work undertaken to modify the procedure for measurement of safrole and/or isosafrole added at the 100 ppm level to ham reported earlier (Zubillaga and Maerker, 1989). The modifications provide a method that is more sensitive and more suitable for field use than the earlier procedure which was more labor intensive and contained steps that gave rise to imprecision.

MATERIALS & METHODS

SAFROLE (97%) and isosafrole (97%) were purchased from Aldrich Chemical Company (Milwaukee, WI). Isosafrole as purchased, contained 15% *cis*-isomer and 85% *trans*-isomer as determined by GC and NMR. Celite 545 was purchased from Fisher Scientific (Malvern, PA). Other chemicals were of reagent grade quality. Domestic cooked or smoked ham was bought in local retail stores. All solvents were "distilled-in-glass" grade and were degassed for HPLC by vacuum filtration through a 0.2 μm filter.

To prepare stock solutions safrole or isosafrole (50 mg weighed accurately) was diluted to 10 mL total volume with hexane:ethyl acetate (95:5, v/v).

Appropriate amounts of the stock solutions of safrole and isosafrole, or mixtures, were diluted to 25 mL with hexane:ethyl acetate (95:5, v/v) so that the final model solutions contained 25 μg to 500 μg of the compounds. An aliquot of each model solution (20 μL) was analyzed by HPLC.

The dry column extraction procedure described previously (Zubillaga and Maerker, 1989) was applied with one modification—stock solutions of safrole and isosafrole were diluted with hexane:ethyl acetate (95:5, v/v) so that 100 μL of the solution added to 5g ham contained appropriate amounts (25 to 500 μg) of safrole, isosafrole, or mixtures. The eluting solvent was hexane:ethyl acetate (95:5, v/v), and the first 25 mL of eluate were collected. An aliquot of the eluate (20 μL) was analyzed by HPLC.

The ham used to prepare the "blank" column had no added compounds. After the column was prepared in the usual manner, 25 mL of the appropriate model solution was added to the surface of the powder. The amounts of safrole, isosafrole, or mixtures of the two, in the model solution were exactly equal to those used to add to the ham in the corresponding experimental extraction above. The eluting solvent was hexane:ethyl acetate (95:5, v/v), and the first 25 mL of "blank" eluate were collected. An aliquot of the eluate (20 μL) was analyzed by HPLC.

Analytical HPLC was performed with an Autochrom, Inc. (Milford, MA) model M500A pump, a model M112 CIM for three-solvent gradients, and a model M320 gradient workstation software/hardware. The system included a Spectroflow model 773 UV detector (Kratos Analytical Instruments, Westwood, NJ) a Rheodyne (Cotati, CA) model 7125 injector with a 20 μL loop, and a Spectra-Physics (Piscataway, NJ) SP 4290 computing integrator. Normal phase separations were performed on columns having the indicated particle size: 3.9 mm × 30 cm μ-Porasil, 10μ (Waters Associates, Milford, MA); 4.0 mm × 25 cm Chromegasphere Si60, 5μ or 3μ (ES Industries, Marlton, NJ) and 4.6 mm × 25 cm Chromega 84% Silica/Alumina, 5μ (E.S. Industries). Flow rates were 1 mL/min for all columns except the 3μ Chromegasphere Si60 where the flow rate was 0.7 mL/min. HPLC analysis was achieved with a gradient solvent system. The mobile phase consisted of hexane:ethyl acetate (95:5, v/v) (solvent A) and isopropanol (solvent B). At injection the solvent was 100% A. After 7 min the solvent composition changed linearly over a period of 3 min to 50% A and 50% B. This ratio was maintained for 5 min and was then changed linearly to 100% A during the following 5 min. Elution of added material occurred within 5 min after injection. The UV detector was set at 215 nm or at 287 nm.

Percent recovery of safrole and/or isosafrole was calculated by comparison of the compound HPLC peak areas of experimental meat sample eluates and "blank" eluates.

RESULTS & DISCUSSION

REVERSED PHASE HPLC has been employed previously to measure safrole in spices with the use of a UV detector (Archer, 1988) and to determine safrole and isosafrole in alcoholic drinks with fluorimetric detection (Currò et al., 1987). In the current work where safrole, and isosafrole when present, was isolated in association with neutral lipids, normal phase HPLC was required to separate the desired compounds from the lipids. Three different normal phase stationary phases were tested. A 10μ particle size μ-Porasil column gave excellent separation of the aromatic compounds from the triglycerides; however, resolution of safrole and isosafrole was not attainable. A 5μ Chromegasphere Si60 column was also highly effective in separating the desired compounds from the neutral lipids and also resolved safrole and isosafrole satisfactorily. Finally, a 5μ Chromega 84% Silica/Alumina column also separated the triglycerides well, resolved the *cis* and *trans* isomers of isosafrole, and gave slightly better resolution of safrole and isosafrole than did the Si60 column.

Sassafras wood contains safrole but not isosafrole (Guenther, 1949), and therefore isosafrole should not be found in ham exposed to smoke from this wood. However, safrole could isomerize to isosafrole at elevated temperatures; therefore isosafrole was included in these studies. Recognition of the geometric isomers of isosafrole was not an objective of our study. Most of the work reported here was carried out either on the Si60 or the 84% Silica/Alumina column.

Table 1—Recovery of safrole and isosafrole from experimental ham samples[a]

Compound	Amount added: Concentration (ppm)	No. of samples	Percent recovery
Safrole	100	8	94 ± 1.5
Safrole	25	2	102 ± 3.5
Safrole	10	4	98 ± 7.5
Safrole	1	3	91 ± 2.0
Isosafrole	100	11	98 ± 5.5
Isosafrole	25	2	100 ± 2.3
Isosafrole	10	8	97 ± 2.8
Isosafrole	5	2	100 ± 2.0

[a] Detection: 215 nm, 287 nm.

Table 2—Recovery of safrole/isosafrole mixtures from experimental ham samples[a,b]

Ratio safrole/isosafrole	Added concentrations (ppm)	Percent recovery vs blank
90/10	100	94 ± 2.9
90/10	10	93 ± 2.5
90/10	5	96 ± 3.0
50/50	100	100 ± 6.0

[a] Detection: 215 nm, 287 nm.
[b] In each case six samples were analyzed.

Safrole and isosafrole concentrations were determined either at 215 nm or at 287 nm. Plots of UV response vs concentration for both compounds and at both wavelengths were linear between 4 ng and 400 ng per 20 μL, corresponding to concentrations of 1–100 ppm of spike in 5g meat samples.

Recovery of the added compounds from meat was measured in 25 mL eluate by comparison with 25 mL of "blank" eluate. Differences between safrole/isosafrole content of experimental sample and "blank" eluates represent losses due to volatilization and manipulation. Differences between model solution and "blank" eluate are due to unavoidable compound retention by the dry column.

Recoveries of safrole or isosafrole from experimental ham samples are shown in Table 1. Most of the samples listed were analyzed on the 5μ Si60 column with detection at 215 nm or 287 nm. Recoveries of safrole or isosafrole compared to "blank" are excellent down to an addition level of 5 ppm and drop off somewhat for the 1 ppm safrole sample. Limits of detection clearly are lower than those at which the compounds were measured, but the value of such lower level detection in the demonstration of sassafras smoking is minimal. Some spices that might be used in ham processing might contribute these compounds at that level.

Recovery of safrole/isosafrole mixtures from experimental ham samples is presented in Table 2. These samples were analyzed on 5μ 84% Silica/Alumina columns which could resolve the components of the mixture. Detection was at 215 nm or 287 nm. Recovery is calculated in comparison to "blank" eluate. Most of the experimental samples contained 90% safrole and 10% isosafrole (w/w), a composition that might be generated if some of the safrole in sassafras smoke isomerizes. Recoveries were about the same over the range investigated (5–100 ppm added materials). The ratio of safrole to isosafrole in the dry column eluate was about the same as that of the experimental sample.

The modified method has distinct advantages over the earlier one. Sensitivity is increased from 100 ppm to 5 ppm without loss of precision, analysis time per sample is decreased sharply, and a liquid chromatograph is the only instrument required.

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